Cell-cycle Disturbance and Induction of Programmed Death by New Formamidine Analogs of Daunorubicin

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Abstract. *Background/Aim: Structural modifications of daunorubicin are an important way to change its anticancer activity. For this reason, formamidinodaunorubicins have been synthesized. The present study was undertaken to determine and compare the in vitro effects of daunorubicin and its new formamidine derivatives on human acute leukemia MOLT-4 and ML-1 cells. Materials and Methods: The experiments were performed on human acute lymphoblastic leukemia MOLT-4 cells and human acute myeloblastic leukemia ML-1 cells. The study was conducted using flow cytometry and light microscopy methods. Results: The various patterns of temporary changes in the cell cycle and DNA fragmentation, as well as the extent of mitotic catastrophe, apoptosis, and necrosis, were determined. The anti-leukemic activities of the new daunorubicin analogs were weaker than that of daunorubicin. Conclusion: The influence of these anthracyclines on cell-cycle progression, DNA damage, and induction of mitotic catastrophe and cell death depended on the agent and its concentration, the time interval after application, and the cell line used. The structural modifications of daunorubicin were responsible for the different cytotoxic effects of the two formamidinodaunorubicins.*

The anthracycline antibiotic daunorubicin is one of the most potent anticancer drugs used in cancer chemotherapy (1, 2). Nevertheless, its clinical efficacy is limited by many serious side-effects, including cardiotoxicity and development of multi-drug resistance (3-6). One of the new strategies for cancer treatment is the design and synthesis of new analogs of daunorubicin, *e.g.* formamidinodaunorubicins. In the chemical structure of these derivatives of daunorubicin, the amidine group is bound to the daunosamine moiety at C-3' position (Figure 1) and contains the rest of the cyclic amines with gradually increasing ring size (7, 8). Literature data suggest that these formamidinodaunorubicins can be promising anticancer agents (9, 10).

The cell cycle and programmed death are accepted to be closely linked and regulated (11-14). In normal cells there is a subtle balance between processes of proliferation and programmed death. It is postulated that the decision of cell-cycle-progression or death can result from a conflict of signals that induce mitotic division and cell-cycle arrest. Mitotic failure can be caused by DNA damage. Mitotic catastrophe, a process preceding cell death, can trigger apoptotic and necrotic processes in the cell. Based on the complex interactions between the regulatory pathways of the cell cycle and programmed death, the cell makes a final decision to divide or to die.

Understanding the action of anthracyclines requires knowledge of the cell-cycle phase at which the cell activates its suicide machinery in response to DNA-damaging agents and their ability to induce programmed death (12). However, only few reports on cell-cycle specificity in terms of DNA damage by different chemotherapeutic drugs have been published (1, 13, 15). Available information on the influence of the new formamidine derivatives of daunorubicin on cell death induction is scarce (16). Moreover, as far as we are aware of, there are no published data on the effects of the formamidinodaunorubicin compounds on cell-cycle progression, DNA damage, and triggering of mitotic catastrophe in human acute leukemia cells.

The aim of the present study was to determine and compare the effects of daunorubicin and new formamidine analogs containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group, on human acute lymphoblastic leukemia MOLT-4 cells and human acute myeloblastic leukemia ML-1 cells. The influence of these two new derivatives of daunorubicin on the cell cycle, DNA fragmentation, and induction of mitotic catastrophe and programmed death of these leukemia cells was analyzed.

Materials and Methods

Cells. Human acute lymphoblastic leukemia MOLT-4 cells and human acute myeloblastic leukemia ML-1 cells were purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). Cells

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were cultured in RPMI-1640 medium (Gibco BRL Life Technologies, Warsaw, Poland) supplemented with 10% fetal calf serum (Gibco BRL Life Technologies), 2 mM L-glutamine (Sigma Aldrich, Poznań, Poland), and antibiotic antimycotic solution (AAS; Sigma Aldrich). AAS contained 20 units of penicillin, 20 μg streptomycin and 0.05 μg amphotericin B. Every third day, cells were passaged. Cells were maintained at 37˚C in a humidified atmosphere of 95% air and 5% CO_2 (HERAcell incubator; KendroLab, Warsaw, Poland).

Chemicals. Daunorubicin, and its morpholine derivative (DAUFmor) and hexamethyleneimine derivative (DAUFhex) were synthesized at the Institute of Biotechnology and Antibiotics (Warszawa, Poland). Daunorubicin, DAUFmor and DAUFhex were dissolved in aqua pro injectione (Polpharma, Starogard Gdański, Poland) at a concentration of 0.5 mM and stored in the dark at −20˚C. All solutions were freshly-prepared directly before treatment of MOLT-4 and ML-1 cells.

Anthracycline concentrations and cell treatment. After a dilution of cell suspension to a density of 15×10^4 cells/ml medium, MOLT-4 and ML-1 cells were treated with the tested anthracycline agents. MOLT-4 cells were exposed to daunorubicin, DAUFmor, and DAUFhex at three concentrations of 25 nM, 50 nM, and 75 nM, respectively, and ML-1 cells to the action of these anthracycline agents, at concentrations of 75 nM, 150 nM, and 225 nM, respectively. The control material consisted of untreated MOLT-4 and ML-1 cells.

Analyses of leukemia cells after anthracycline application. Temporary changes occurring in MOLT-4 cells and ML-1 cells were determined at 24, 48, and 72 h after the application of daunorubicin, DAUFmor and DAUFhex. At these three time intervals, the cell-cycle distribution and DNA fragmentation were analyzed using a flow cytometric terminal dUTP nick-end labeling (TUNEL) method, and leukemia cells undergoing mitotic catastrophe, apoptosis, and necrosis were observed under a light microscope.

TUNEL assay. TUNEL assay was performed using the APO-BrdU Kit (Calbiochem, San Diego, USA) according to the manufacturer's instructions. Briefly, cells were fixed in 1% formaldehyde, on ice for 15 min, and then stored at −20˚C in 70% ethanol until analysis. Cells were removed from the ethanol and washed prior to DNA labeling. 5-Bromo-2'-deoxyuridine 5'-triphosphate nucleotides (Br-dU) were incorporated onto exposed 3'-hydroxyl DNA ends by incubation with deoxynucleotidyl transferase TdT for 60 min at 37˚C. Following DNA labeling, the cells were stained with FITC-labeled anti-BrdU in the dark for 30 min at room temperature. Finally, the cells were incubated with RNase/propidium iodide (PI) solution and then incubated in the dark for 30 min at room temperature. The fluorescence of MOLT-4 and ML-1 cells stained with FITC-conjugated antibody to BrdU and PI was detected by a FACS Calibur flow cytometer (Becton Dickinson, New Jersey, USA). CellQuest Pro software (Becton Dickinson, New Jersey, USA) was used for acquisition and analysis of data. Temporary changes in the frequency of all leukemia cells (TUNEL-positive and TUNEL-negative) and cells expressing DNA strand breaks (TUNEL-positive) observed in the particular phases of the cell cycle, and in the frequency of polyploid cells, as well as temporary alterations in the sub-G1 population, were analyzed.

Cell morphology. Leukemia cell suspension, diluted in phosphate buffered saline (BioMed, Lublin, Poland), containing approximately 2×10^5 cells, was added into a cytospin chamber and cytocentrifuged for 6 min at 100 × *g* and at 4˚C. After air drying, the prepared cytospins

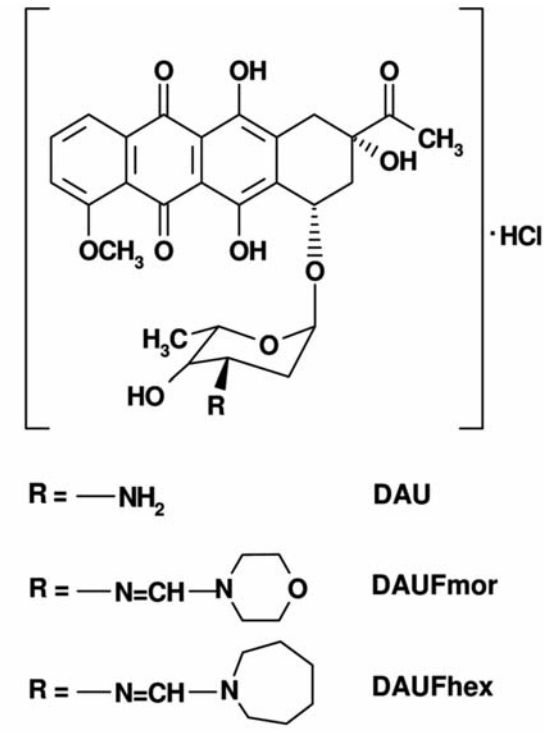

Figure 1. *Chemical structures of daunorubicin (DAU) and its two derivatives containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group.*

were fixed in methanol at room temperature, for 15 min. Cells were stained using the Wright-Giemsa method according to the manufacturer's instruction (Aqua-Med, Łódź, Poland). Morphological observations were performed under a light microscope at a magnification of ×1000. Based on the morphology of leukemia cells, the frequency of MOLT-4 and ML-1 cells undergoing mitotic catastrophe, apoptosis, and necrosis was determined.

Statistical evaluation. All experiments were repeated three times with duplicate or triplicate determinations. All the data are presented as the mean values±standard deviation. Statistical analyses were performed using STATISTICA 10 (StatSoft, Kraków, Poland). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant differences multiple range test. Results were considered as significant when the *p*-value was less than 0.05.

Results

Effects of daunorubicin, DAUFmor and DAUFhex on MOLT-4 and ML-1 cells. The effects of the parent antibiotic daunorubicin and its two analogs, DAUFmor and DAUFhex, on the cell cycle, DNA fragmentation, mitotic catastrophe and programmed death of acute leukemia MOLT-4 and ML-1 cells, were assessed. The different patterns of temporary changes in the frequency of leukemia cells in the different phases of the cell cycle, the frequency of polyploid cells and the sub-G_1 population (Figure 2), the frequency of cells with DNA strand breaks (Figure 3)

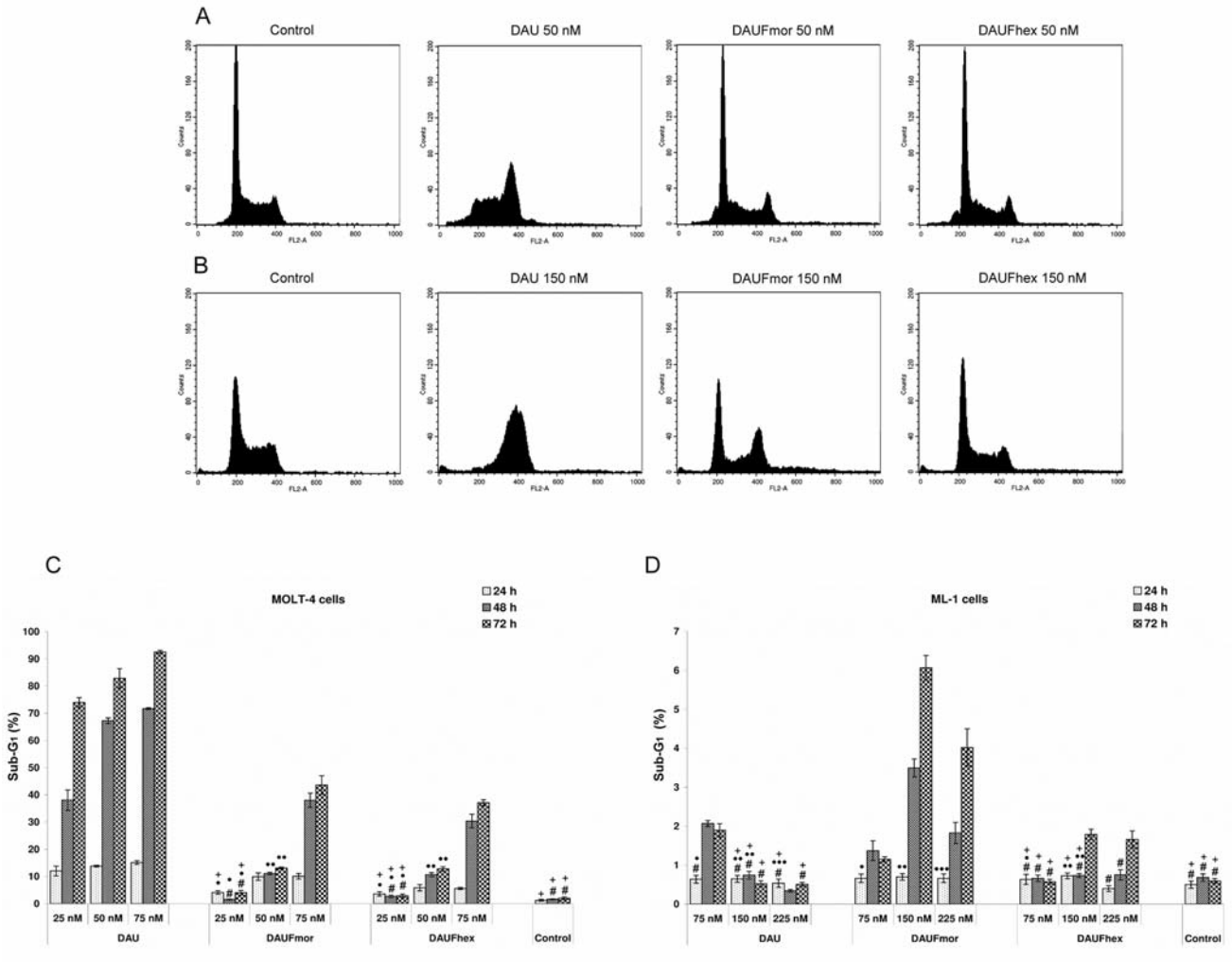

Figure 2. *continued*

and TUNEL-positive cells found in the different phases of the cell cycle (Figure 4), as well as the extent of mitotic catastrophe, apoptosis, and necrosis (Figure 5), were determined throughout a 72 h period after the application of daunorubicin and formamidinodaunorubicin agents.

Cell-cycle disturbance. Daunorubicin and its formamidine derivatives distinctly affected the cell-cycle distribution of the acute leukemia cells. Daunorubicin caused a blockage of ML-1 cells in the G_2/M phase of the cell cycle observed throughout 72 h period, but a decrease of the frequency of MOLT-4 cells in this phase at 48 h and a 72-h after the parent antibiotic application. Moreover, an increase of the sub-G_1 population was found at 24 h after the exposure of MOLT-4 cells to the action of daunorubicin. The cell cycle was disrupted to a lesser extent by DAU analogs than the parent anthracycline. Among the daunorubicin derivatives, DAUFmor appeared to be more active in causing cell-cycle disruption in ML-1 cells than DAUFhex. Polyploid leukemia cells were observed after the application of daunorubicin, DAUFmor and DAUFhex. However, the highest frequency of polyploid cells was observed after the exposure of ML-1 cells to the action of DAUFmor (Figure 2).

DNA damage. Daunorubicin induced the formation of DNA strand breaks in human acute leukemia MOLT-4 and ML-1 cells to a higher degree than did its analogs, DAUFmor and DAUFhex. Out of the two derivatives, DAUFmor appeared to be more active in triggering the formation of DNA strand breaks than DAUFhex (Figure 3).

DNA strand breaks in cell-cycle phases. After the application of daunorubicin and its two analogs, DAUFmor and DAUFhex, DNA damage was not found to be cell-cycle phase specific. The results indicate that daunorubicin, DAUFmor and DAUFhex induced DNA fragmentation in a cell-cycle phase-independent manner (Figure 4). These three anthracyclines caused DNA

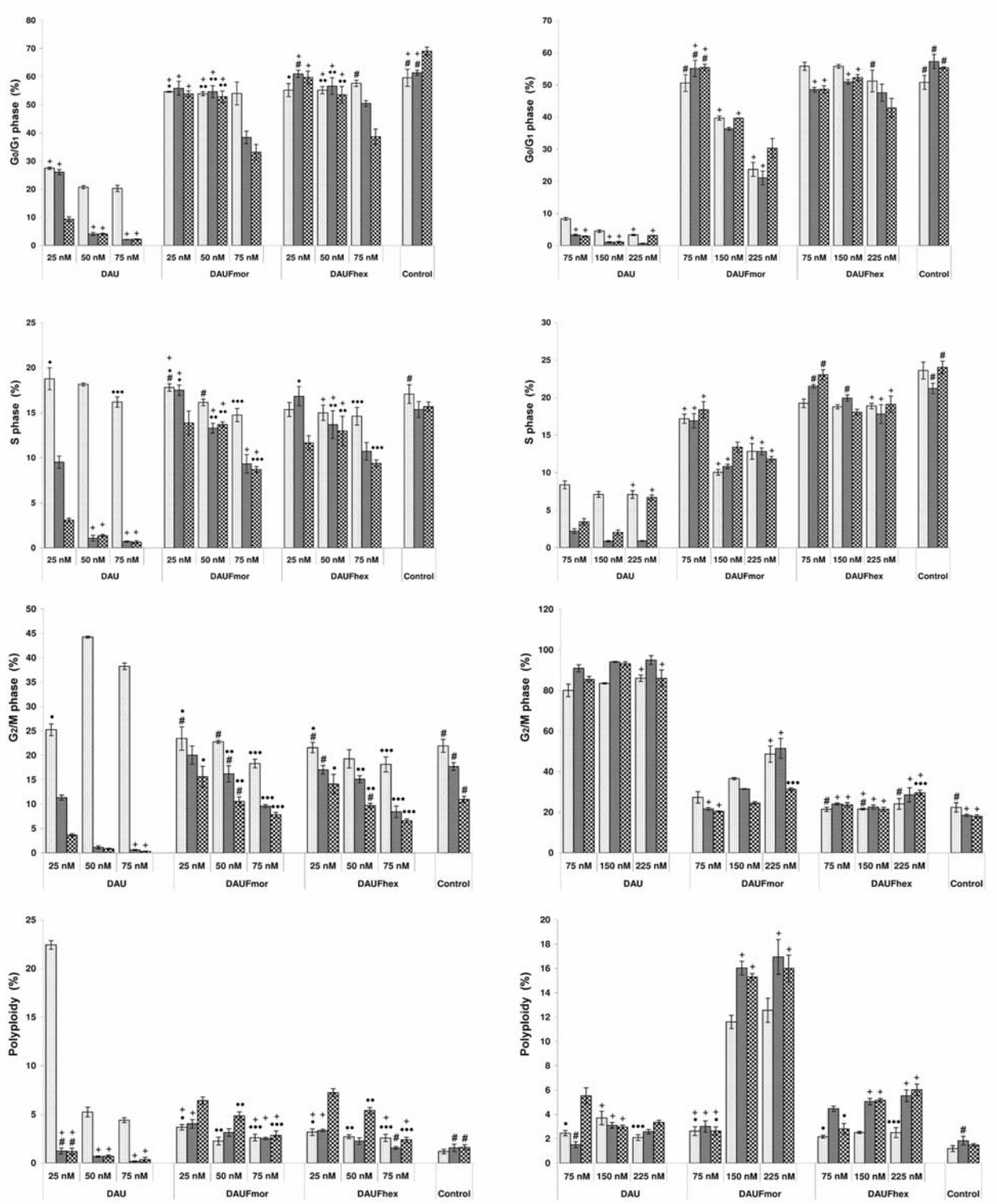

Figure 2. *Effects of daunorubicin (DAU), and its new derivatives containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group, on the cell-cycle distribution of MOLT-4 and ML-1 leukemia cells. Representative histograms of the cell cycle of MOLT-4 (A) and ML-1 (B) cells analyzed at 24 h after their exposure to the action of DAU, DAUFmor and DAUFhex. The peaks observed in the channel FL2-A show leukemia cells, respectively, in G_1 phase at a value of 200; and in G_2/M phase of the cell-cycle at a value of 400. The compartment below a value of 200 represents the sub-G_1 region which can contain apoptotic bodies or fragments of necrotic cells. The frequency of MOLT-4 (C) and ML-1 cells (D) in the particular phases of the cell cycle. Values not significantly different at p>0.05 according to the Tukey's multiple range test: ●, ●●, ●●●. Between the groups of leukemia cells treated with the anthracycline agents; #compared to controls; +between the time points.*

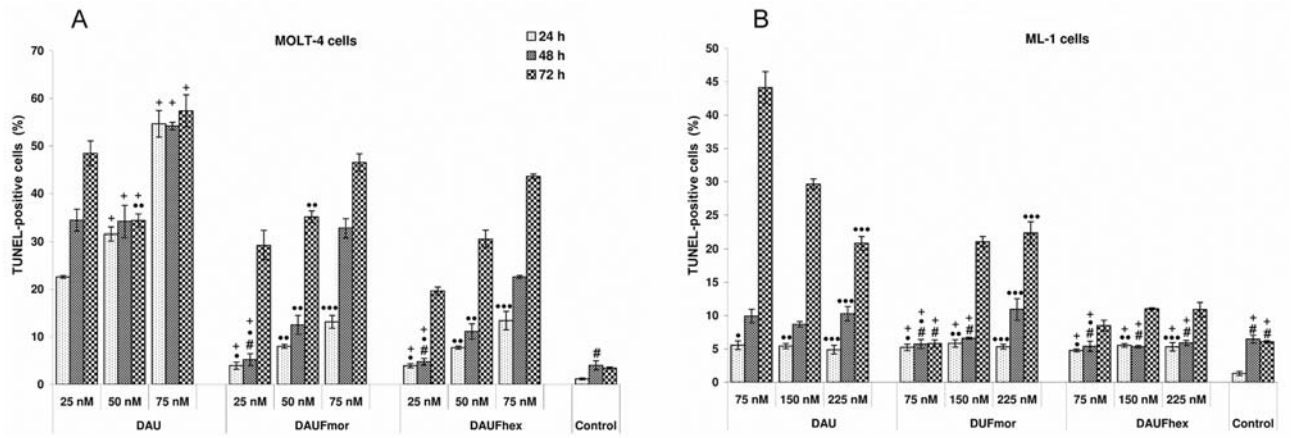

Figure 3. *In situ detection of DNA strand breaks in MOLT-4 and ML-1 cells after application of daunorubicin (DAU), and its new derivatives containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group, using flow cytometry APO-BrdU™ assay. Temporary changes in the frequency of terminal dUTP nick-end labeling (TUNEL)-positive MOLT-4 (A) and ML-1 (B) cells, determined throughout 72 h after DAU, DAUFmor and DAUFhex application. Values not significantly different at p>0.05 according to the Tukey's multiple range test: ●, ●●, ●●●. Between the groups of leukemia cells treated with the anthracycline agents; #compared to controls; +between the time points.*

strand breaks in G_0/G_1, S and G_2/M phases of the cell cycle of MOLT-4 and ML-1 cells. Daunorubicin affected DNA fragmentation of MOLT-4 and ML-1 cells to a greater degree than its analogs. In all phases of the cell cycle, a lower frequency of ML-1 cells with DNA strand breaks was found than MOLT-4 cells.

Effects of daunorubicin and formamidinodaunorubicins on cell morphology. After the exposure of MOLT-4 and ML-1 cells to the action of daunorubicin, DAUFmor and DAUFhex, the leukemia cells underwent mitotic catastrophe, apoptosis and necrosis (Figure 5). DAUFmor induced mitotic catastrophe in leukemia cells to a higher degree than did DAU and DAUFhex, and this was especially observed in ML-1 cells. Among the tested anthracyclines, daunorubicin caused an increase of the frequency of both apoptotic ML-1 cells and necrotic MOLT-4 cells to a greater degree than did its analogs.

Discussion

The effects of daunorubicin and its two formamidine analogs on cell-cycle disturbance, and induction of mitotic catastrophe and programmed death of human acute leukemia cells were compared. The anti-leukemic potential of daunorubicin, DAUFmor, and DAUFhex towards MOLT-4 and ML-1 cells depended on the tested compound and its concentration, the time interval after the anthracycline application, and the cell line used.

The structural modifications of daunorubicin were responsible for different anti-leukemic activities of its two analogs against MOLT-4 and ML-1 cells. Daunorubicin appeared to be more cytotoxic than its formamidine derivatives. Of the two tested daunorubicin analogs, DAUFhex, containing a seven-membered hexamethyleneimine ring with a CH_2 group in the γ-position, was less active than DAUFmor, containing a six-membered morpholine ring with an oxygen heteroatom in the γ-position in the formamidine group. The presence of an oxygen heteroatom in the morpholine ring is probably responsible for forming an additional hydrogen bond with proton-donating sites, due to the presence of the free electron pairs on the oxygen atom (9, 10, 16, 17). A number of different mechanisms have been proposed for the cytotoxic action of daunorubicin, including reactive oxygen species production, DNA-damage induction, interactions with DNA topoisomerases I and II, and cell membranes, and also triggering of programmed cell death (18-21). Nevertheless, the mechanisms of anticancer activity of the anthracyclines are not yet completely understood. Available information on the cytotoxicity of formamidinodaunorubicins is scant (9, 10, 16, 17).

There is a general consensus that DNA is the main target for a large number of anticancer agents (22, 23). The anthracycline antibiotics, including daunorubicin, are accepted to be DNA-damaging agents (18). After the application of daunorubicin and its new derivatives, DNA strand breaks were observed at all phases of the cell cycle. Thus, daunorubicin and its formamidine analogs, DAUFmor and DAUFhex, are non-cell-cycle phase-specific agents. Moreover, MOLT-4 cells appeared to be more sensitive to triggering of DNA fragmentation by the tested anthracycline compounds than ML-1 cells, and daunorubicin caused DNA degradation to a greater degree than did DAUFmor and DAUFhex. These results confirmed the data published by Ciesielska *et al*. in which daunorubicin induced DNA damage in leukemia L1210 cells to a greater extent than did DAUFmor and DAUFhex (24).

It is known that DNA degradation can affect cell-cycle progression and inductor of mitotic catastrophe and

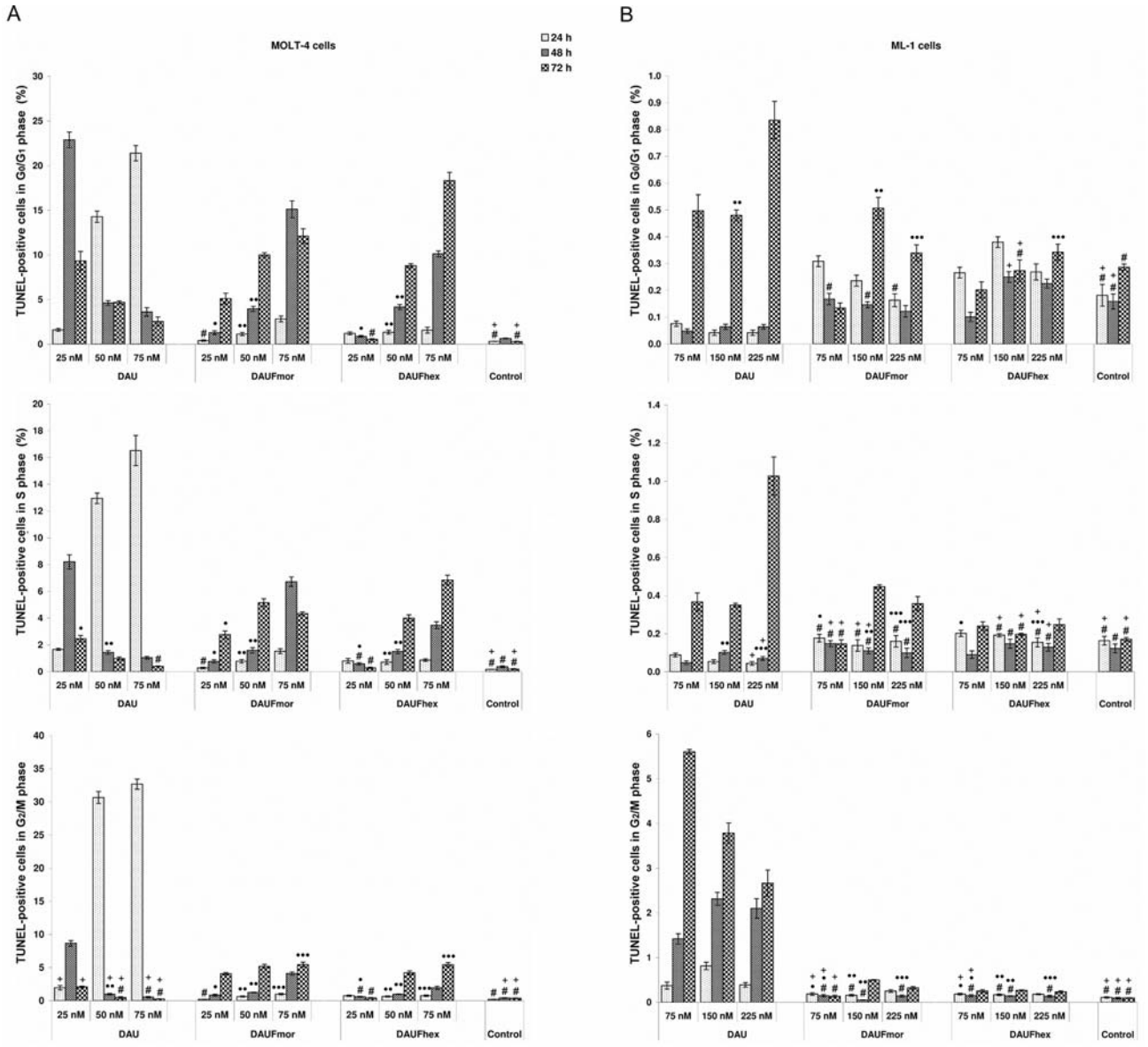

Figure 4. *Temporary changes in the frequency of terminal dUTP nick-end labeling (TUNEL)-positive MOLT-4 (A) and ML-1 (B) cells in the particular phases of the cell cycle, determined throughout the 72 h after daunorubicin (DAU), and its new derivatives containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group application. Values not significantly different at p>0.05 according to the Tukey's multiple range test: ●, ●●, ●●●. Between the groups of leukemia cells treated with the anthracycline agents; #compared to controls; +between the time points.*

programmed cell death (20, 25, 26). The present study shows that DAUFmor and DAUFhex triggered DNA fragmentation, which could be responsible for abnormal mitosis, mitotic catastrophe and cell death shown by the morphology of the treated leukemia cells.

Mitotic catastrophe is accepted not to be a separate mode of cell death but rather a process preceding cell death through apoptosis or necrosis (27). Cells undergoing mitotic catastrophe originate from abnormal, delayed or abrogated mitosis (26). One of the most prominent morphological features of mitotic catastrophe is the presence of multi-nucleated giant cells possessing abnormal nuclei with incomplete nuclear condensation and micronuclei. Mitotic catastrophe can also be manifested by multipolar meta- or anaphase, accidental distribution of condensed chromosomes, or the formation of nuclear envelopes around individual clusters of missegregated uncondensed chromosomes or chromosome fragments (28). Taking into account these morphological features of the human

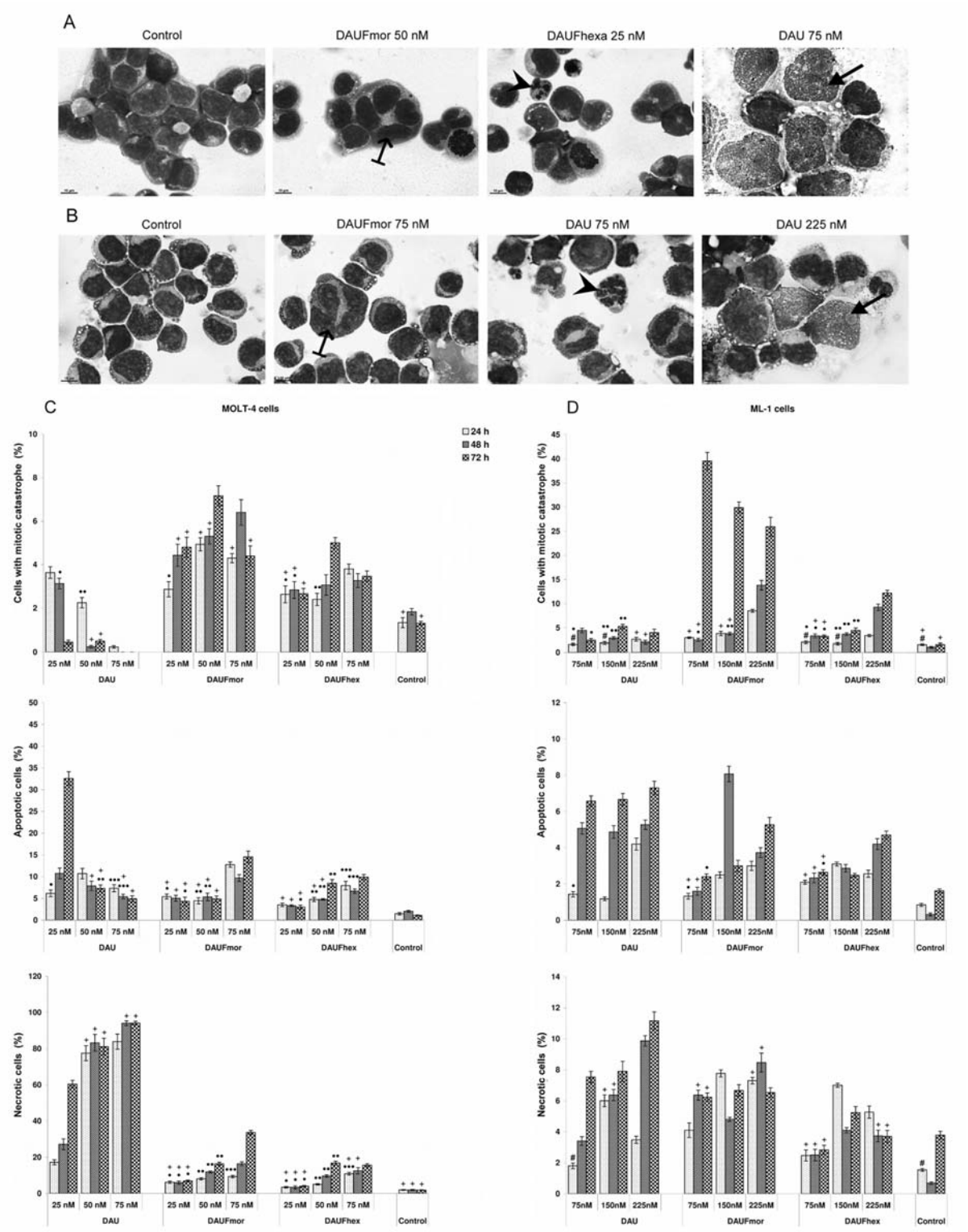

Figure 5. *Morphology of MOLT-4 (A) and ML-1 (B) cells at 24 h after their exposure to the action of the three anthracyclines, daunorubicin (DAU), and its new derivatives containing either a morpholine moiety (DAUFmor) or a hexamethyleneimine moiety (DAUFhex) in the amidine group. MOLT-4 and ML-1 cells, stained with the Wright-Giemsa, were visible under light microscopy. Arrow with bar indicates cells with mitotic catastrophe, arrowhead indicates apoptotic cells, and long arrow indicates necrotic cells. Scale bar – 10 μm. The frequency of MOLT-4 (C) and ML-1 (D) cells undergoing mitotic catastrophe, apoptosis, and necrosis. Values not significantly different at p>0.05 according to the Tukey's multiple range test:* ●, ●●, ●●●. *Between the groups of leukemia cells treated with the anthracycline agents; #compared to controls; +between the time points.*

acute leukemia MOLT-4 and ML-1 cells exposed to the action of daunorubicin and its two formamidine analogs DAUFmor and DAUFhex, noticeable differences in the extent of mitotic catastrophe were found.

Apoptosis and necrosis are the two main types of programmed cell death. The characteristic morphological features of apoptotic cells includes cell shrinkage, condensation of chromatin and nuclear fragmentation. Necrotic cells are characterized by an increased size, cellular membrane disintegration and nuclear degradation. Daunorubicin, DAUFmor, and DAUFhex induced both apoptotic and necrotic death of MOLT-4 and ML-1 cells. The influence of the anthracyclines, including daunorubicin and doxorubicin, on the induction of apoptotic and necrotic death of HL-60 and Jurkat cells was also reported by Dartsch *et al*. (20).

In conclusion, it can be generally stated that the cytotoxic effects of daunorubicin, DAUFmor and DAUFhex on MOLT-4 and ML-1 cells depended on their different chemical structures. These are the first data showing the *in vitro* effects of new formamidine derivatives of daunorubicin, DAUFmor and DAUFhex, on cell-cycle perturbation, DNA damage, induction of mitotic catastrophe and on triggering morphological alterations in human acute lymphoblastic MOLT-4 and myeloblastic ML-1 leukemia cells undergoing programmed death.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

Acknowledgements

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References

1 Hortobagyi GN: Anthracyclines in the treatment of cancer. An overview. Drugs *54*: 1-7, 1997.
2 Nadas J and Sun D: Anthracyclines as effective anticancer agents. Expert Opin Drug Discov *1*: 539-548, 2006.
3 Birtle AJ: Anthracyclines and cardiotoxicity. Clin Oncol (R Coll Radiol) *12*: 146-152, 2000.
4 Minotti G, Menna P, Salvatorelli E, Cairo G and Gianni L: Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev *56*: 185-229, 2004.
5 Peng X, Chen B, Lim CC and Sawyer DB: The cardiotoxicology of anthracycline chemotherapeutics: translating molecular mecha-nism into preventative medicine. Mol Interv *5*: 163-171, 2005.
6 Minotti G and Sarvazyan N: The anthracyclines: When good things go bad. Cardiovasc Toxicol *7*: 53-55, 2007.
7 Oszczapowicz I, Wąsowska M, Oszczapowicz J, Owoc A, Dominiczak E, Wietrzyk J and Opolski A: New derivatives of anthracycline antibiotics, method of their synthesis, pharmaceutical agents containing them, and their use. Polish Patent 210494, 2005 (in Polish).
8 Wąsowska M, Oszczapowicz J, Oszczapowicz I and Owoc A: New method of synthesis of anthracycline antibiotic derivatives. Polish Patent Application P. 381654, 2006 (in Polish).
9 Wąsowska M, Oszczapowicz I, Wietrzyk J, Opolski A, Madej J, Dzimira S and Oszczapowicz J: Influence of the structure of new anthracycline antibiotics on their biological properties. Anticancer Res *25*: 2043-2048, 2005.
10 Wąsowska M, Wietrzyk J, Opolski A, Oszczapowicz J and Oszczapowicz I: Effect of structural modification of anthracyclines on the ability to overcome drug resistance of cancer cells. Anticancer Res *26*: 2009-2012, 2006.
11 Evan GI, Brown L, Whyte M and Harrington E: Apoptosis and the cell cycle. Curr Opin Cell Biol 7: 825-834, 1995.
12 Halicka HD, Seiter K, Feldman EJ, Traganos F, Mittelman A, Ahmed T and Darzynkiewicz Z: Cell cycle specificity of apoptosis during treatment of leukaemias. Apoptosis *2*: 25-39, 1997.
13 Mazur L, Augustynek A, Deptała A, Halicka HD and Bedner E: Effects of WR-2721 and cyclophosphamide on the cell-cycle phase specificity of apoptosis in mouse bone marrow. Anticancer Drugs *13*: 751-758, 2002.
14 Alenzi FQ: Links between apoptosis, proliferation and the cell cycle. Br J Biomed Sci *61*: 99-102, 2004.
15 Côme MG, Skladanowski A, Larsen AK and Laurent G: Dual mechanism of daunorubicin-induced cell death in both sensitive and MDR-resistant HL-60 cells. Br J Cancer *79*: 1090-1097, 1999.
16 Stojak M, Mazur L, Opydo-Chanek M, Łukawska M and Oszczapowicz I: *In vitro* induction of apoptosis and necrosis by new derivatives of daunorubicin. Anticancer Res *33*: 4439-4444, 2013.
17 Stojak M, Mazur L, Opydo-Chanek M, Łukawska M and Oszczapowicz I: Effects of structural modifications of daunorubicin on *in vitro* antileukemic activity. Anticancer Res *32*: 5271-5278, 2012.
18 Gewirtz DA: A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol *57*: 727-741, 1999.
19 Laurent G and Jaffrézou JP: Signaling pathways activated by daunorubicin. Blood *98*: 913-924, 2001.
20 Dartsch DC, Schaefer A, Boldt S, Kolch W and Marquardt H: Comparison of anthracycline-induced death of human leukemia cells: programmed cell death versus necrosis. Apoptosis *7*: 537-548, 2002.
21 Szuławska A and Czyż M: Molecular mechanisms of anthra-cyclines action. Postepy Hig Med Dosw *60*: 78-100, 2006 (in Polish).
22 Bischoff G and Hoffmann S: DNA-binding of drugs used in medicinal therapies. Curr Med Chem *9*: 312-348, 2002.
23 Palchaudhuri R and Hergenrother PJ: DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. Curr Opin Biotechnol *18*: 497-503, 2007.
24 Ciesielska E, Studzian K, Wąsowska M, Oszczapowicz I and Szmigiero L: Cytotoxicity, cellular uptake and DNA damage by daunorubicin and its new analogues with modified daunosamine moiety. Cell Biol Toxicol *21*: 139-147, 2005.
25 Mansilla S, Piña B and Portugal J: Daunorubicin-induced variations in gene transcription: commitment to proliferation arrest, senescence and apoptosis. Biochem J *372*: 703-711, 2003.
26 Mansilla S, Bataller M and Portugal J: Mitotic catastrophe as a consequence of chemotherapy. Anticancer Agents Med Chem *6*: 589-602, 2006.
27 Portugal J, Bataller M and Mansilla S: Cell death pathways in response to antitumor therapy. Tumori *95*: 409-421, 2009.
28 Mazur L, Stojak M, Opydo-Chanek M and Niemeyer U: Mitotic catastrophe induction in U937 cells by oxazaphosphorines. Acta Biol Cracov Zool *51*: 17-22, 2009 (in Polish).

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